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# Effects on bone tissue in ewes (Ovies aries) and their foetuses exposed to PCB118 and PCB153

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# PREFACE

This report is a graduate project in Environmental Toxicology at Uppsala University. The laboratory part of the project was carried out at the Department of Orthopedics at Uppsala University Hospital. The bone material was obtained from the Norwegian School of Veterinary Science, Oslo, Norway.

I would like to thank my supervisor Associate Professor Monica Lind at the institute of Environmental Medicine, Karolinska Institutet and Associate Professor Jan Örberg at the Department of Environmental Toxicology for their advice and help in dark times. PhD Arno Gutleb at the Norwegian School of Veterinary Science, Oslo, Norway for providing the bone material and background information of the animals. Professor Sune Larson for the help with the breaking of the bones. Special thanks to Assistant Professor Katrin Lundstedt-Enkel for guidance in the statistical jungle. Finally I like to thank Denise my laboratory partner for support through out the entire project, for letting me steel her pictures and for giving me the insight that I am plastic and changeable in contrast to stone.

# SUMMARY

PCBs were first introduced on the market in the year of 1929 and in the 1960s the discovery was made that it had accumulated in almost every environmental matrix subjected for testing.
The decrease of the seal population coupled to accumulation of PCBs along with poisoning accidents in humans led to a successive ban from 1972 to 1995 when a complete ban was enforced. From the Second World War there has been an increase in age-adjusted osteoporotic-fractures accompanied by an increase of organochlorines in the environment. The question whether a connection between organochlorines, as PCBs, and osteoporosis does exist has been lifted. Osteoporosis is a big health issue in Sweden related to some 70 000 fractures every year accompanied by a cost estimated to around 4.6 billion SEK.

Bone is a highly changeable tissue and hormones are one among many things that is controlling bone tissue metabolism. PCBs are thought to be endocrine disrupting and thus have the potential to disturb the development and homeostasis in bone. In the appendicular skeletal bones (the extremities) there are two types of bone; trabecular and cortical bone. Bone tissue is constantly subjected to a remodelling process, bone is resorbed and produced, which involves both types of bone but the trabecular bone is considered to be more metabolic active than the cortical bone.
Effect-studies on bone tissue alterations, where links has been found with PCBs have for example been performed on mink, polar bears, goat, turtle, humans and rat etc.

The aim of this present study was to elucidate if two of the most frequently found PCBs in nature, the dioxin-like mono-ortho PCB118 and non dioxin-like di-ortho PCB153, would cause changes on bone composition and strength in sheep ewes and their foetuses.
A total of 55 ewes where allocated in 4 groups; reference, control (corn oil 0.1ml/kg body wt/day), PCB118 (49 µg/kg body wt/day) and PCB153 (98 µg/kg body wt/day). The drug was administered orally every third day starting at the day of conception. Cross contamination caused a mixed exposure scenario in all but the reference group which was considered “clean” as levels of PCB was below the detection limit.
The distribution of the concentration between the two PCB congeners was the same for all three groups of individuals (foetus male, foetus female and adult female) with average sum of the two congeners lowest in the control group and highest in the PCB153 group. The least cross contaminated group was the PCB153 group with the lowest level of PCB118 and the highest of PCB153. The PCB118 group had the highest concentration of PCB118 and the control group had the lowest of PCB153.

The ewes were euthanised approximately one week prior calculated delivery and femur was dissected free from the ewes and foetuses. Bone composition were measured using peripheral Quantitative Computed Tomography (pQCT) on metaphyseal measure point (18 % of the total bone length from a reference point near the epiphyseal growth plate at the knee joint for the foetuses and 4.8 % for the ewes) and diaphyseal measure points (40 %, 50 % and 60 % of the total bone length for the foetuses and 40 % and 50 % for the ewes). Bone strength was evaluated using three point bending test at 50 % of the total bone length. Statistical analysis where performed using ANCOVA with Bonferroni/Dunn post hoc. test with a significance level of 0.0083, correction was made for weight.

There were no significant differences found between the groups of ewes.

Several differences from the pQCT measurements were obtained in the foetus bone material. Trabecular Bone Mineral Content (BMC) was almost 30 % lower in the PCB118 group compared to the control group and the reference group at the metaphyseal measure point in the male group. Trabecular Cross Section Area (CSA) was 19 % lower in the

PCB153 group compared to the control group and total Bone Mineral Density (BMD) was 17 % higher in the PCB153 group compared to the reference group at the metaphyseal measure point in the female group.

Both female and male groups had lower marrow cavity area with as much as 24 % in the PCB153 groups compared to control group and reference group, at the diaphyseal measure point. Among female foetuses, there was a clear difference at the mid diaphyseal measure point between the PCB153 group and the control group. The variables cortical and total Bone Mineral Density (BMD) and cortical thickness was higher in the PCB153 group compared to the control group and endosteal circumference together with marrow cavity was lower compared to the control.
In conclusion there was a difference in the response to the treatment between the sexes in the foetus group and cortical bone was indicated to be more affected than trabecular bone.

# INTRODUCTION

Polychlorinated biphenyl (PCB) is a widely distributed contaminant found in almost every environmental matrix subjected for testing [1]. Banned for many years it still poses a highly current threat to our environment. Effect has been seen in many different species (polar bear, mink, goat, rat etc) [2, 3, 4, 5] and on many physiological functions (reproductive, immune, neurological) [1, 6, 7]. Bone growth and metabolism is controlled by sex hormones. PCB has the potential to disturb this process since some of the PCB congeners mimic sex hormones. Changes in bone composition have been measured in experimental exposure setups [3, 4, 5] as well as in natural occurring exposure scenarios of PCBs [2]. The increasing levels of organochlorines since the Second World War is accompanied by an increasing rate of age-adjusted osteoporotic- related fractures in man. This has lead to the suspicion that there might be a connection between that exposure to PCB and to other organochlorines and osteoporosis [8]. In Sweden, the total yearly cost for the more than 70000 osteoporotic-related fractures is estimated to be 4.6 billion SEK [9].

## Bone

### Bone-formation, growth and remodelling

The vertebral skeleton consists of two parts; appendicular (the extremities) and axial (non extremities) [10]. There are two types of bone tissues; cortical and trabecular. Cortical bone is compact bone forming the outer shell of the bone. Trabecular bone is more metabolic active than cortical bone and has a big surface area due to its spongy structure [11]. There are four bone cells operating within the bone; *osteoblast, osteoclasts, osteocyts and lining-cells* [12] (fact box 1). The appendicular bone is formed by endochondral ossification. A model of the bone is formed in cartilage by chondrocyte-cells. The ossification process starts in the centre of the cartilage bone model where the chondrocytes increase in size and die, leaving behind an empty space covered by a thin layer of calcified matrix. This is called the first ossification centre. Blood vessels invade the area bringing osteoblast that starts ossification from the central part of the cartilage-model proceeding in the length axis towards the epiphyseal parts (for description of the nomenclature of the different parts of the femur bone (fig. 1) [13].

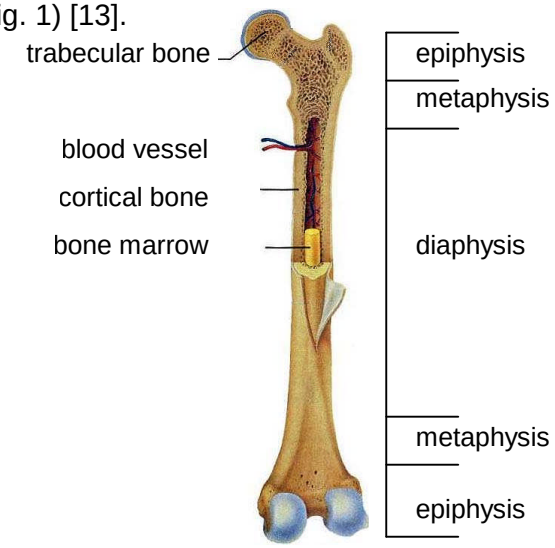


**Figure 1.** Schematic picture of a femur bone visualising the different parts of the bone. Modified from [37]

**Fact box 1**

*Osteoblasts*- Production and mineralization of the protein based bone matrix [13]. They operate as a communication centre mediating signals for the osteoclasts. They fill the lacuna created by the osteoclasts with new bone matrix [15]. Approximate lifetime is three month [16].

*Osteoclasts*- Multinuclear cells that resorp the bone matrix. Containing lots of lysosomal vesicles they resorp bone, giving rise to small lacuna. Signal that starts the resorption is received through the osteoblasts [15]. Approximate lifetime is two weeks [16].

*Osteocytes*- A mature form of osteoblast that is deposited in newly formed bone matrix [10, 15, 17, 16]. They are in contact with each others and other cells on the surface of the bone through extensions of their cell membrane. Function is not clearly understood and is described ambiguous in the literature (mechanosensors for functional adaptation of the bone, ion-changing function, involved in resorption/production of bone) [11, 17, 13, 18].

*Lining-cells*- A mature form of osteoblasts [17, 16, 15] placed on the surface of bone tissue that is not undergoing resorption/production [11]. Function is vaguely described in the literature as taking part in the process of bone resorption. They are said to be clearing the path for the osteoclast by removing the unmineralized collagen covering the surface of bone [17].

A second ossification centre for longitudinal growth is formed at the epiphyseal ends later in the development of the bone. Chondrocytes at different development stages constitutes the epiphyseal plate, or growth plate. At the final stage the chondrocytes is hypertrophied, increasing in height 6-10 times, and calcifies the surrounding matrix before they die [14]. The ossification process then proceeds as described above and the epiphyseal plate remains for as long as longitudinal bone growth occurs [13].

Growth in the diametric dimension is at the diaphyseal part of appendicular bone is described as production of new bone on the outside and resorption from the inside. The resorption gives rise to the marrow cavity. At the epiphyseal part the growth is somewhat different. To allow both length and diametric growth at the same time, resorption needs to occur on the outside at the ends of the epiphysis and production on the inside [11].

Trabecular bone is found mostly at the second ossification centre, at the distal ends and cortical bone more towards the middle of the bone where the bone has had longer time to mature. Hormonal action on bone and the relationship to osteoporosis is an intense research area. Bone constantly undergoes a remodelling process of resorption and new formation of bone. When the remodelling process of bone starts to favour resorption the osteoporosis develops.

Both osteoblast and osteoclasts have receptors for estrogen and estrogen is believed to decrease the activity of the osteoclasts [16] and decrease their lifetime [19]. Estrogen has also been shown to be necessary for the closure of the epiphyseal plate [10, 14]. Estrogen therapy has been shown to improve the Bone Mineral Density (BMD) in postmenopausal women [15]. Except from hormonal regulation of bone, nutritional factors as vitamin A, C, D, and K and calcium and phosphorus mineral are also very important.

## PCB

### History

In 1929 the first PCBs produced in industry were placed on the market. The use soon spread to multiple applications because of their stable chemical and physical properties. PCBs were for example used in capacitors, insulators, softeners, glue and in caulking compounds. It was not until the 1960s that the discovery was made that PCBs hade accumulated in almost every environmental matrix subjected for testing. The decrease of the seal population along with poisoning accidents in humans led to a first ban for all use in open systems in Sweden in 1972. This was followed by a stop for all new use of the compounds in 1978 and in 1995 a complete ban even for use of older products containing PCBs [1].

### Properties

PCBs are in fact a group of 209 related congeners. The molecular structure consists of a biphenyl with chlorine substituents. The difference lies in the number and positions of the chlorine substituents. Therefore, in nature PCB is found as a mixture of the different congeners. In general the PCBs are soluble in fat and are not readily degradable, allowing them to readily accumulate in biological matrixes [21]. The more chlorine-substituted PCBs are more lipophilic than the lesser chlorine-substituted ones [1]. 14 of the 209 congeners constitutes a group that is referred to as “dioxin like” [22].They have a structure similar to the well documented toxic compounds PolyChlorinated DibenzoDioxins (PCDD) and PolyChlorinated DibenzoFurans (PCDF). The most toxic forms within these two groups are 2,3,7,8-tetrachloro-dibenzo-*p*-dioxin and 2,3,7,8-tetrachloro-dibenzofuaran [21].
Like PCDD and PCDF these 14 dioxin-like PCB congeners have a co-planar structure and a fairly high affinity to an intracellular receptor called arylhydrocarbon receptor (AhR). Toxic effects of dioxins are mediated through the AhR which is also the pathway for the estrogen degradation [23].

### PCB118 and PCB153

2,3',4,4',5-pentaclorobiphenyl (PCB118) is one of the dioxin-like PCBs whereas 2,2',4,4',5,5'-hexaxhlorobiphenyl (PCB153) is not [22] (see fig.2 for chemical structure). *In vitro* studies have ascribed PCB118 and PCB153 week anti-estrogen characteristics [23, 25, 26]. In the same study on human-breast-cancer-cells [23], PCB118 and PCB153 have been shown to elevate the metabolism of estrogen. PCB153 but not PCB118 was shown to be able to interact with the estrogen receptor and block the estrogen mediated response. PCB118 but not PCB153 was shown to decrease the aromatase activity (enzyme that transforms testosterone to estrogen).
The antiestrogenic characteristics of PCB118 are something that would be expected since it has the ability to bind to AhR and then up-regulate the degradation pathway of estrogen. Diverging results exists for PCB153; an *in vivo* study on goat, suggested PCB153 to be estrogenic rather than anti-estrogenic [4]. Both PCB118 and PCB153 are considered to be endocrine disrupting chemicals since they have the potential to interfere with the endocrine system.
A study on human from the late 1980s regarding half-life of PCBs in the human body, indicate that PCB153 is somewhat less degradable than PCB118. 100-300 days was measured for PCB118 and 338 days for PCB153 [27]. Half-life for PCB118 in air has been documented to be approximately 2 months and in water 6 years. The corresponding time for PCB153 has been documented to be 8 months and 6 years, respectively [28]. Both PCB118 and 153 belongs to the top list over most frequently found PCBs in biotic and abiotic matrixes [24].

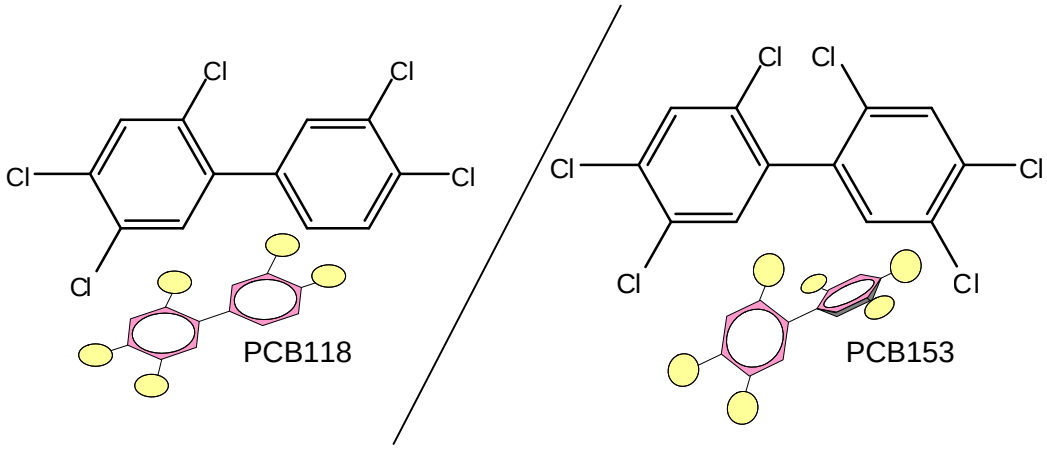


**Figure 2.** Chemical structures of co-planar dioxin-like PCB118 and the non-co-planar non-dioxin-like PCB153. Modified from www.chemfinder.com

### Levels

Male human serum concentration of PCB118 has been reported to be in average 41.9 ng/g lipid and PCB153 to be 291.3 ng/g lipid (N=115) [29]. Female human serum concentration has been reported to have a median value for PCB118 of 43 ng/g lipid and for PCB153; 223 ng/g lipid (n=225) [30]. Since the late 1970s the concentration of PCBs in human milk in Sweden has been monitored. Total concentration of PCBs declined from 1977-1985 but in 1989 the trend ceased and the total PCB concentration settled around 0.6 µg/g lipid. The ratio between PCB118 and PCB153 in human milk is approximately 1:5 and with a slight decline of PCB118 and increase of PCB153 during the measured period 1977-1989 expressed as per cent of total PCBs [31].

## Effect studies on bone and PCBs

### Human

A comparative study between women on the east and west coast of Sweden did show a higher incidence of osteoporotic related fractures for the east coast women. The women in the study were all married to fishermen. The study did not include other factors that could

have affected the result as smoking habits etc [32]. In another epidemiologic study, which included both females and males from the Swedish west and east costs, a negative correlation between plasma level of PCB153 and bone mineral density (BMD) in forearm bones was observed. After correction for age and body mass index, however, no such correlation could be found [33].

**Polar bears**

A study over time on skulls from polar bear *(Ursus maritimus*) using dual x-ray absorptiometry did show a connection between BMD and level of PCB. Skulls collected in 1892-1932 (before organochlorines) had higher BMD compared with skulls from the time period 1966-2002. Tissue samples from the latter period were analysed for total PCB and a negative correlation between the level of PCB and BMD in skulls from sub adult male specimens was found [2].

**Seals**

A study of the connection between suspected environmental load of organochlorines and BMD was performed on male Baltic grey seals (*Halichoerus grypus*). Measurements were performed on radius and mandible bones using pQCT. Radius bones were collected from the time period 1965-1985 (a period of high concentrations of organochlorines in the environment) and from 1986-1997 (a period with decreasing concentrations of organochlorines in the environment). The mandible bones were collected from the same two periods as the radius bones and also from the time period between 1850 and 1955 (a period with very low concentrations of organochlorines in the environment). Cortical BMD in the mandible bones was highest in the early time period and successive lower in the two following time periods. Trabecular BMD of the radius was higher in the specimens collected 1986 – 1997 than in those collected 1965 – 1985 [34].

**Goats**

Exposure to PCB153 (98µg/kg body wt/day) was in a study on goat offspring shown to alter bone composition. Pregnant ewes were exposed from day 60 of gestation until delivery. The offspring where euthanised after 9 months during which they had lactated for 6 weeks. Measurements were performed on femur with peripheral Quantitative Computed Tomography (pQCT). Compared with control animals the exposed animals had higher trabecular BMD, smaller Total Cross Sectional Area (the total area of the bone in a cross sectional, CSA) Marrow Cavity (the area of the cavity) and lower Moment of Resistance (a theoretical value of the strength of the bone). The higher value of trabecular BMD was interpreted as an estrogenic effect [4].

**Turtles**

Diamondback terrapin turtles (*Malaclemys terrapin) were* exposed to PCB126 and skulls were examined with CT- scan and ashing. Exposed animals had a lower BMD and organic content in skulls compared to controls [35].

**Sheep**

Lambs (*Ovies aries*) were exposed in utero and during lactation to a mixture of PCB118 and PCB153 and then euthanised at an age of approximately two month. Bone composition and strength were evaluated with pQCT and tree point bending test. The lambs were allocated in four groups, low and high dose from each PCB congener based on tissue analysis of PCB concentration. Significant difference was only found in the PCB118 groups high and low exposure. The pQCT variables Polar Moment of Inertia and Polar Moment of Resistance were higher on the 35 % and 50% measure points of the total bone length. These variables are calculated variables from the pQCT measurements connected to the strength of the bone [36].

### Mink

Mink was fed with a concentration of PCB126 that was 36 times higher than the concentration in fish and 7 times higher than the concentration in the bird eggs that are the natural diet for free ranging minks. Minks from the group treated with PCB126 developed porous bone in the upper and lower jaw bone along with loosening of teeth's. This was not seen on the control animals [3].

### Rats

Sprague-Dawley rats injected intraperitoneally with PCB126 (64 µg/kg body wt a total of five injections) showed changes on humerus on several structural bone variables measured with pQCT as well as changes in strength of the bone with biomechanical testing. Exposed animals had a reduced length of the femur and smaller total CSA, cortical CSA, trabecular CSA and greater cortical thickness and higher trabecular BMD, compared with controls. The maximum torque at failure along with the average stiffness was significantly smaller in exposed animals than in the controls [5].

## THE AIM

The aim of this study was to determine possible effects of exposure to PCB118 and PCB153 on bone composition and strength in pregnant ewes and their offspring.

# MATERIAL AND METHODS

## Experimental animals

Pregnant ewes (*Ovies aries,* a Norwegian breed *Dala*) and their foetuses were used to evaluate the possible toxic effects of PCB118 and PCB153 on bone tissue. Ewes and unborn lambs were euthanised one week prior calculated delivery date of the lambs. The ewes were housed at the University of Life Science in Ås, Norway. The animals were kept outside during the summer months and inside during the winter. Apart from this study the ewes had been taking part in one experimental setup in the years 2004-2005. They were then given either PCB118 or PCB153 under the gestation period 145 days. However single events of cross contamination occurred and created a mixed exposure scenario.

## Exposure and groups

In this study the ewes were allocated by block randomization in 4 groups; reference, control (corn oil 0.1 ml/kg body wt/day), PCB118 (49 µg/kg body wt/day and PCB153 (98 µg/kg body wt/day). Using oral administration and corn oil as vehicle the groups got their treatment every third day starting on the first day of gestation continuing until euthanisation (138 days from mating). The groups were treated from late 2005 and ending in spring 2006.
A total of 55 ewes were used in the study and they carried a total 65 male and 53 female lambs. Some ewes had three or even four lambs but only two from each ewe where used for bone measurements. This left 56 male and 46 female lambs for analysis. The ewes varied in age from 2-7 years. The different groups were separated both indoor and outdoor so that no cross contamination through faeces could occur. However, due to erroneous handling of animals, single events of cross contamination occurred resulting in a mixed exposure scenario in all groups but the reference group. Therefore, the group planned to represent a control group has to be considered as low contaminated. The residue levels in adipose tissue of PCB 118 and PCB 153 in foetuses and ewes are presented in tables 1, 2 and 3 [38].

## Length measurements

A calliper was placed between the middle of the joints with the calliper parallel to the length axis of the bone. When the 40 % and 50 % point was assigned the hip joint was the reference with the calliper placed in the middle of the joint. For adult bones an analogue calliper with an accuracy of 0.1 mm was used. For foetus bones a digital calliper with accuracy 0.01 mm was used (see fig.4).

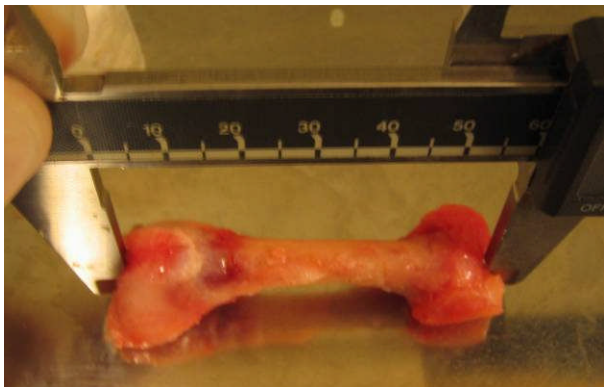

**Figure 3.** Measurement of the total bone length with a slide calliper. The calliper was placed in the middle of the joints with the calliper parallel to the length axis of the bone

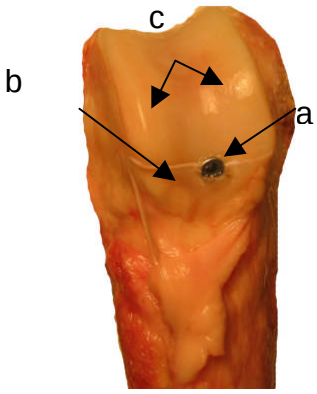


**Figure 4.** Knee joint of the femur bone showing the reference screw (a), inserted at *linea intercondylaris* (b), *trochlea ossis femori* (c)

## Reference point

For the pQCT measurements a reference point was needed (fig.4 and 5) to enable comparison between bones. On the anterior side of the knee there are two ridges named *Trochlea ossis femori*. Between these ridges there is a transverse line (*linea intercondylaris*). This morphological structure was chosen as the reference point and a wood screw (size: 2.5 x12 mm) was fully inserted with the tip slightly pointing towards the knee joint.

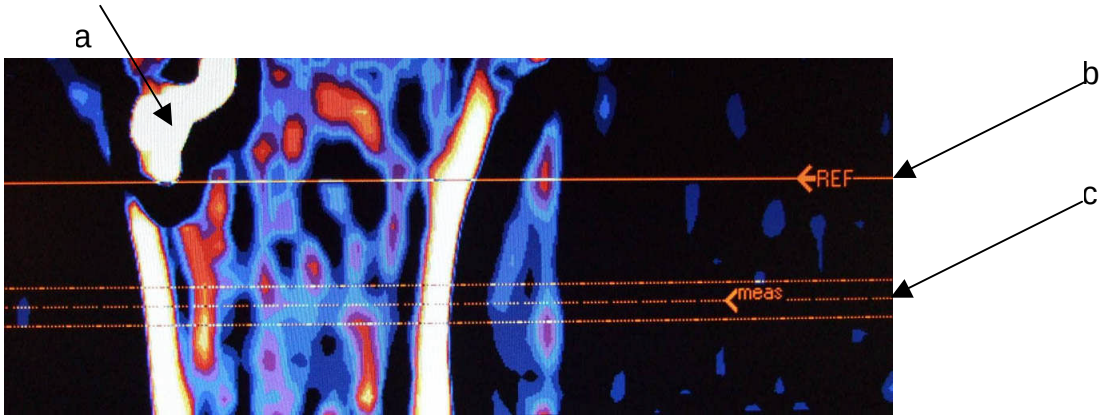


**Figure 5.** The Scan View in the pQCT software of an adult bone showing the reference screw (a), the reference line adjusted against the screw (b) and the measure point 4.8 % of the total bone length from the screw (c)

## Packing of bones

After the reference screw was inserted the bones were packed using gauze bandage moistened with ringer solution (pH: 7.4, 0,3 g Tris, 9 g NaCl, 0.215 g $CaCl_2$, 0.4 g KCl och 2.05 ml 1 M HCl per 1 l $H_2O$) covered with polythene. This was done to hinder dehydration of the bones (fig.6).

**Figure 6.** The bones were moistened with ringer solution and covered with gauze bandage and polythene.

## Storage of bones

The bones were stored in a freezer (-18 degrees) and before measurements in pQCT they were defrosted in a fridge approximately 24 h before measurements. After measurements in the pQCT they were once again placed in a freezer until biomechanical bending test could be performed. Before bending test was performed they were again defrosted in a fridge (24 h).

## peripheral Quantitative Computed Tomography

For detection of differences in bone composition between the different groups a computerized X-ray machine, pQCT (Stratec XCT 960A software v. 5.20; Norland Stratec Medizintechnik, Pforzheim, Germany) was used. The accuracy of the pQCT was evaluated once a week using a standard phantom. To make sure that reproducibility was secured, one bone from each material (foetus and adults) was scanned 10 times and repositioned between measurements to obtain the Coefficient of Variation. This was done in the same manner as the final measurements.

**Metaphyseal measure point**

To choose the trabecular measure point, three criteria were used.

**1** Enclosed circle of cortical bone with a bone mineral density ≥ 690 mg/cm$^3$.
**2** Large trabecular area.
**3** Small variations in trabecular area and trabecular bone mineral density between individuals and along length axis of the bone.

Results from five bone specimens from ewes and four from foetuses were used for the determination of the metaphyseal measure point.

The variables determined at the metaphyseal measure point were; total BMC mg/mm (Bone Mineral Content), total BMD mg/cm$^3$ (Bone Mineral Density), trabecular BMC mg/mm, trabecular BMD mg/cm$^3$, total CSA mm$^2$ (Cross Sectional Area of both trabecular and cortical bone), trabecular CSA mm$^2$, periosteal circumference mm (the outer circumference of the bone, a mathematical model named "the circular ring model" was used to describe the shape of the bone as circular).

**Metaphyseal measurements of foetal bones**

Trabecular bone was measured by scanning at 18 % of the total bone length proximal of the reference point (the wood screw). The scout view function in the program was used to place the reference line at the edge of the screw facing the diaphysis. For the analysis peel mode 2, contour mode 1, true filter, threshold 270 mg/cm$^3$, inner threshold 500 mg/cm$^3$ and voxel size B (590 µm) were used.

**Metaphyseal measurements of bones from ewes**

Trabecular bone was measured by scanning at 4.8 % of the total bone length proximal of the reference point. The procedure and settings were the same as for the measurements of bones from fetuses apart from the voxel size. In this case voxel size A (689 µm) was used.

**Diaphyseal measure point**

The variables determined at the diaphyseal measure points were; total BMC mg/mm, total BMD mg/cm$^3$, total CSA mm$^2$, cortical BMC mg/mm, cortical BMD mg/cm$^3$, cortical CSA mm$^2$, cortical thickness mm (from the circular ring model), periosteal circumference mm, endosteal circumference (the inner circumference of the bone, from the circular ring model), moment of resistance mm$^3$ (theoretical calculated value of the bone strength), marrow cavity mm$^2$.

**Diaphyseal measurements of foetal bones**

For measurements of cortical bone the light point function in the program was used and scanning was performed at 40, 50 and 60 % of the total bone length. For the analysis peel mode 2, contour mode 1, threshold 690 mg/cm$^3$ and voxel size B (590 µm) were used.

**Diaphyseal measurements of bones from ewes**

The procedure and settings were the same as for the measurements of bones from fetuses apart from the voxel size. In this case voxel size A (689 µm) was used. Measurements were performed at 40 % and 50 % of the total bone length.

**Positioning in the pQCT**

The bones were positioned with the knee joint pointing in to the machine, as straight as possible. Both the foetus bones and the adult bones were leaned on the hip ball side of the bone.

## Three point bending test

To evaluate the bone strength a three point bending test was performed using a MTS minibionix servohydraulic testing machine. The axial capacity of the machine is 10 000 N and loading speed was set to 1mm/sec. The load was applied vertically at the 50 % measure point. For the adult bones the distance between the supporting points was 100 mm and 30 mm for the foetus bones. The bones were dorsally positioned in the machine. From a Load-Displacement curve four variables were considered, each representing different aspects of the bones ability to withstand stress. The variables used was “Load at failure” (N, the load at the moment of failure), “max Stiffness” (N/mm, the elastic property of the bone), “Energy Absorption” (N*mm, the energy required to break the bone) and “Displacement” (mm, the deformation of the bone up till the breakage point). A typical Load/Displacement curve is represented in figure 7.

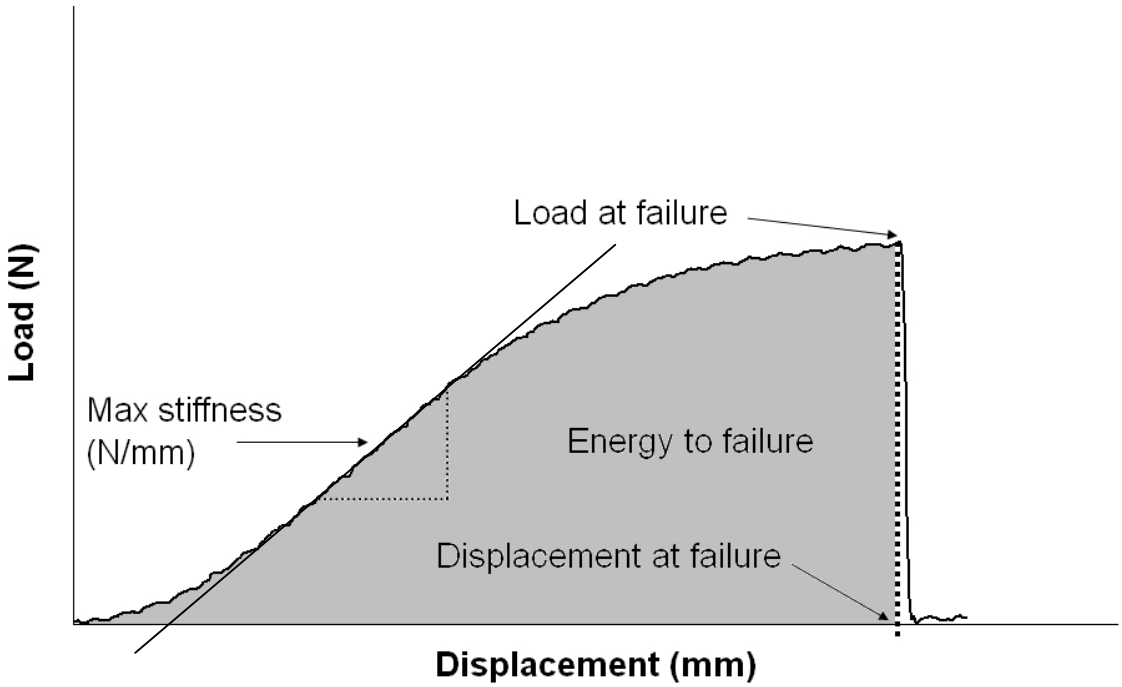


**Figure 7.** An example of a Load-Displacement curve [39]. Load at failure (N): the load at the moment of failure. Max stiffness (N/mm): the slope of the steepest part of the curve reflects the elastic property of the bone. Energy absorption (N*mm): the area under the curve up to the point of failure is the amount of energy needed for breaking of the bone. Displacement (mm): represents the deformation of the bone.

## Statistics

Statistical analysis was performed using StatView 5.0; SAS Institute INC., Cary, NC, USA in which ANCOVA with Bonferroni/Dunn as post hoc with significance level of 0.0083 were used. For the ANCOVA a significance level of 0.05 was used. All data were assumed to have normal distribution if passing D’Agostino & Pearson omnibus normality tests in software program GrapPAD PRISM. Only data that has a normal distribution is allowed in the Statview software when working with the ANCOVA. Therefore interpretation of not Gaussian data is problematic. Adjustment for body weight was done in the ANCOVA. In the adult female group the results from the reference could not bee used in the ANCOVA since weights of the animals were missing.

# RESULTS

## Reproducibility

The Coefficient of Variation from the pQCT measurements varied from 0.19 % (periosteal circumference 50 % measure point) to 3.14 % (moment of resistance 60 % measure point) for the foetuses and for the adult from 0.13 % (periosteal circumference 40 % measure point) to 2.05 % (trabecular BMC 4.8 % measure point). The measurements of the total length of the bone had a coefficient of variation of 0.36 % for the foetuses and 0.31 % for the adults.

## Concentrations in tissue and background facts

The average concentration in fat tissue of the two PCBs, body weight, bone length and additional background information for the three groups (foetus male, foetus female and adult female) are presented in tables 1, 2 and 3. No analyses of fat tissue were performed on the reference groups and no weight was recorded for the ewes.

The bone length in the foetus female group was lower in the PCB118 and PCB153 groups compared with the control group (table 2).

The distribution of the concentration between the two PCB congeners was the same for all three groups of individuals (foetus male, foetus female and adult female) with average sum of the two PCB congeners PCB118 and PCB153 lowest in the control group and highest in the PCB153 group. The lowest average concentration of PCB118 was found in the PCB153 group which also had the highest concentration of PCB153. These treatment groups (PCB153) were thus the least contaminated groups. The PCB118 group had the highest average concentration of PCB118 and the control group had the lowest PCB153 concentration.

**Table 1.** Concentration (mean ± standard deviation) in fat tissue from male foetuses of ewes orally administered PCB118 (49 µg/kg body wt/day) and PCB153 (98 µg/kg body wt/day). Cross contamination created a mixed exposure scenario. Reference was not treated and PCB residue levels in the reference group were below detection limits. Weight, bone length, lipid concentration in analyzed fat tissue, number of days in uterus before euthanisation and number of siblings for each animal are also expressed as an average for each treatment group. NA = Not Analysed, BDL = Below Detection Limit.

| **Foetus Male** | Reference | Control | PCB118 | PCB153 |
|---|---|---|---|---|
| (N) | 17 | 12 | 17 | 10 |
| Weight (kg) | 5.1 ± 1.1 | 5.6 ± 0.8 | 4.8 ± 1.0 | 4.7 ± 0.7 |
| No. of days in uterus | 135 ± 4 | 134 ± 2 | 135 ± 2 | 134 ± 1 |
| Litter size | 2.4 ± 0.87 | 2.2 ± 0.39 | 2.0 ± 0.71 | 1.9 ± 0.32 |
| Bone length (mm) | 88.2 ± 6.1 | 92.5 ± 6.4 | 85.8 ± 5.7 | 85.6 ± 4.9 |
| Lipid conc. of fat tissue (%) | NA | 41 ± 7 | 41 ± 4 | 41 ± 4 |
| PCB118 (µg/g lipid) | BDL | 1.97 ± 0.49 | 6.43 ± 2.04 | 0.52 ± 0.24 |
| PCB153 (µg/g lipid) | BDL | 1.63 ± 0.37 | 2.38 ± 0.89 | 47.5 ± 5.4 |
| PCB118+PCB153 (µg/g lipid) | BDL | 3.59 ± 0.78 | 8.82 ± 2.68 | 48.0 ± 5.6 |

**Table 2.** Concentration (mean ± standard deviation) in fat tissue from female foetuses of ewes orally administered PCB118 (49 µg/kg body wt/day) and PCB153 (98 µg/kg body wt/day). Cross contamination created a mixed exposure scenario. Reference was not treated and PCB residue levels in the reference group were below detection limits. Same letters in superscript indicates differences (ANCOVA with correction for weight Bonfferoni/Dunn post hoc. test significance level of 0.0083).Weight, bone length, lipid concentration in analyzed fat tissue, number of days in uterus before euthanisation and number of siblings for each animal are also expressed as an average for each treatment group. NA = Not Analysed, BDL = Below Detection Limit.

| **Foetus Female** | Reference | Control | PCB118 | PCB153 |
|---|---|---|---|---|
| (N) | 8 | 16 | 8 | 14 |
| Weight (kg) | 4.8 ± 0.97 | 5.0 ± 1.0 | 4.3 ± 0.6 | 4.8 ± 0.6 |
| No. of days in uterus | 134 ± 4 | 134 ± 3 | 134 ± 1.0 | 135 ± 2 |
| Litter size | 2.75 ± 0.71 | 2.38 ± 0.50 | 2.38 ± 0.74 | 2.07 ± 0.47 |
| Bone lenght (mm) | 85.5 ± 6.1 | 88.8 $^{a,b}$ ± 4.6 | 83.6$^{a}$ ± 2.9 | 85.1$^{b}$ ± 4.4 |
| Lipid conc. of fat tissue (%) | NA | 44 ± 4 | 43 ± 5 | 42 ± 6 |
| PCB118 (µg/g lipid) | BDL | 1.97 ± 0.44 | 6.19 ± 1.64 | 0.42 ± 0.07 |
| PCB153 (µg/g lipid) | BDL | 1.50 ± 0.26 | 2.95 ± 1.03 | 47.7 ± 6.11 |
| PCB118+PCB153 (µg/g lipid) | BDL | 3.48 ± 0.64 | 9.14 ± 2.43 | 48.1 ± 6.11 |

**Table 3.** Concentration (mean ± standard deviation) in fat tissue from ewes orally administered PCB118 (49 µg/kg body wt/day) and PCB153 (98 µg/kg body wt/day). Cross contamination created a mixed exposure scenario. Reference was not treated and PCB concentrations in the reference group were below detection limits. Weight, bone length, lipid concentration in analyzed fat tissue, age (both average and min-max) and litter size for each animal are also expressed as an average for each treatment group. NA = Not Analysed, BDL = Below Detection Limit.

| **Adult Female** | Reference | Control | PCB118 | PCB153 |
|---|---|---|---|---|
| (N) | 14 | 14 | 14 | 11 |
| Weight (kg) | NA | 98.5 ± 13.2 | 96.3 ± 12.4 | 98.1 ± 12.5 |
| Age (years) | 4.14 ± 2.18 | 4.43 ± 2.06 | 4.14 ± 1.96 | 4.36 ± 1.96 |
| Age min-max (years) | 2-8 | 2-7 | 2-7 | 2-7 |
| Litter size | 2.36 ± 0.93 | 2.29 ± 0.47 | 2.00 ± 0.78 | 2.00 ± 0.45 |
| Bone length (mm) | 186.6 ± 5.4 | 183.7 ± 8.6 | 182.3 ± 6.5 | 183.5 ± 5.9 |
| Lipid conc. of fat tissue (%) | NA | 75 ± 13 | 80 ± 7.2 | 81 ± 9.2 |
| PCB118 (µg/g lipid) | BDL | 2.98 ± 0.51 | 8.51 ± 1.85 | 0.65 ± 0.19 |
| PCB153 (µg/g lipid) | BDL | 2.75 ± 0.64 | 3.61 ± 1.30 | 59.3 ± 12.2 |
| PCB118+PCB153 (µg/g lipid) | BDL | 5.73 ± 1.06 | 12.1 ± 2.82 | 60.0 ± 12.3 |

## pQCT measurements

### Foetus male

### Methaphysis 18 %

Trabecular BMC was 28 % lower in the PCB118 group (N=17) compared to the reference group (N= 17) and 29 % lower compared to the control group (N=12, P<0.0083) at the metaphyseal 18 % measure point (table 4).

**Table 4.** Results (means ± standard deviation) obtained from the pQCT analysis at the metaphyseal measure point of femur from male foetuses exposed to PCB118 and PCB153. Results from tissue analysis of PCB concentration in the different groups are presented in table 1. Differences were considered significant at 0.0083 (ANCOVA with Bonferroni/Dunn as post hoc). Values sharing the same superscript differ significantly from each other. BMC = Bone Mineral Content, BMD = Bone Mineral Density, CSA = Cross Sectional Area.

| | **Foetus Male (N)** | Reference (17) | Control (12) | PCB118 (17) | PCB153 (10) |
|---|---|---|---|---|---|
| **Metaphysis 18 %** | Total BMC (mg/mm) | 56.5 ± 14.1 | 63.4 ± 8.0 | 56.4 ± 9.6 | 62.8 ± 9.5 |
| | Total BMD (mg/cm$^3$) | 444 ± 86 | 472 ± 74 | 482 ± 111 | 533 ± 61 |
| | Trabecular BMC (mg/mm) | 12.7 ± 2.8$^{a}$ | 12.8 ± 3.7$^{b}$ | 9.07 ± 3.2$^{a.b}$ | 12.2 ± 2.2 |
| | Trabecular BMD (mg/cm$^3$) | 233± 82 | 220 ± 73 | 214 ± 93 | 303 ± 84 |
| | Total CSA (mm$^2$) | 128 ± 23 | 136 ± 20 | 120 ± 22 | 118 ± 13 |
| | Trabecular CSA (mm$^2$) | 59.7 ± 20.1 | 62.1 ± 21.3 | 50.1 ± 22.2 | 42.7 ± 11.2 |
| | Periosteal circumference (mm) | 39.9 ± 3.6 | 41.3 ± 3.0 | 38.7 ± 3.7 | 38.5 ± 2.2 |

### Diaphysis 60 %

No significant differences were found at this measure point (table 5).

**Table 5.** Results (means ± standard deviation) obtained from the pQCT analysis at the diaphyseal measure point 60 % of femur from male foetuses exposed to PCB118 and PCB153. Results from tissue analysis of PCB concentration in the different groups are presented in table 1. Differences were considered significant at 0.0083 (ANCOVA with Bonferroni/Dunn as post hoc). BMC = Bone Mineral Content, BMD = Bone Mineral Density, CSA = Cross Sectional Area.

| | **Foetus Male (N)** | Reference (17) | Control (12) | PCB118 (17) | PCB153 (10) |
|---|---|---|---|---|---|
| **Diaphysis 60 %** | Total BMC (mg/mm) | 66.0 ± 15.6 | 73.7 ± 9.7 | 63.6 ± 10.1 | 65.8 ± 11.3 |
| | Total BMD (mg/cm$^3$) | 646 ± 97 | 679 ± 83 | 650 ± 95 | 699 ± 69 |
| | Total CSA (mm$^2$) | 102 ± 21 | 109 ± 12 | 99.1 ± 17.4 | 94.0 ± 11.7 |
| | Cortical BMC (mg/mm) | 45.7 ± 14.9 | 53.2 ± 12.3 | 44.8 ± 9.8 | 48.9 ± 11.5 |
| | Cortical BMD (mg/cm$^3$) | 918 ± 48 | 938 ± 46 | 927 ± 40 | 943 ± 33 |
| | Cortical CSA (mm$^2$) | 49.3 ± 14.5 | 56.3 ± 10.6 | 48.2 ± 9.5 | 51.7 ± 11.0 |
| | Cortical thickness (mm) | 1.61 ± 0.47 | 1.82 ± 0.41 | 1.62 ± 0.34 | 1.80 ± 0.37 |
| | Periosteal circumference (mm) | 35.7 ± 3.7 | 37.0 ± 1.9 | 35.2 ± 3.2 | 34.3 ± 2.2 |
| | Endosteal circumference (mm) | 25.6 ± 3.9 | 25.5 ± 3.5 | 25.0 ± 4.1 | 23.0 ± 2.2 |
| | Moment of resistance (mm$^3$) | 99.1 ± 37.6 | 115 ± 30.7 | 97.4 ± 26.4 | 103 ± 26.8 |
| | Marrow cavity (mm$^2$) | 53.2 ± 16.6 | 52.8 ± 15.1 | 51.0 ± 15.5 | 42.3 ± 8.01 |

**Diaphysis 50 %**

No significant differences were found at this measure point (table 6).

**Table 6.** Results (means ± standard deviation) obtained from the pQCT analysis at the diaphyseal measure point 50 % of femur from male foetuses exposed to PCB118 and PCB153. Results from tissue analysis of PCB concentration in the different groups are presented in table 1. Differences were considered significant at 0.0083 (ANCOVA with Bonferroni/Dunn as post hoc). BMC = Bone Mineral Content, BMD = Bone Mineral Density, CSA = Cross Sectional Area.

| | **Foetus Male (N)** | Reference (17) | Control (12) | PCB118 (17) | PCB153 (10) |
|---|---|---|---|---|---|
| **Diaphysis 50%** | Total BMC (mg/mm) | 60.8± 14.5 | 65.8± 9.2 | 60.1± 9.3 | 61.6± 10.7 |
| | Total BMD (mg/cm$^3$) | 623± 85 | 631± 83 | 644 ± 92 | 677± 68 |
| | Total CSA (mm$^2$) | 97.8± 20.4 | 105± 13 | 94.8± 17.3 | 90.8± 11.3 |
| | Cortical BMC (mg/mm) | 41.5± 12.7 | 45.6± 11.5 | 42.6± 7.9 | 45.1± 11.1 |
| | Cortical BMD (mg/cm$^3$) | 914± 41 | 933± 42 | 935 ± 38 | 947± 34 |
| | Cortical CSA (mm$^2$) | 45.0± 12.2 | 48.5± 10.6 | 45.4± 7.5 | 47.3± 10.2 |
| | Cortical thickness (mm) | 1.49± 0.36 | 1.57± 0.38 | 1.56± 0.29 | 1.66± 0.35 |
| | Periosteal circumference (mm) | 34.9± 3.7 | 36.2± 2.1 | 34.4± 3.3 | 33.7± 2.2 |
| | Endosteal circumference (mm) | 25.5± 3.6 | 26.4± 3.4 | 24.6± 4.2 | 23.3± 2.2 |
| | Moment of resistance (mm$^3$) | 94.4± 31.4 | 109± 31 | 94.7± 22.9 | 97.5± 28.7 |
| | Marrow cavity (mm$^2$) | 52.8± 15.0 | 56.3± 15.2 | 49.5± 15.5 | 43.5± 8.4 |

**Diaphysis 40 %**

Cortical BMD was 5 % higher in the control group (N=12) compared to the reference group (N= 17) and 5% higher in the PCB153 group (N=10) compared to the reference group, P<0.0083)

Marrow Cavity was 24 % lower in the PCB153 group (N=10) compared to the control group (N=12) and the 21 % lower than in the reference group (N=17, P<0.0083) (table 7). The control group data for the marrow cavity variable did not follow Gaussian distribution.

**Table 7.** Results (means ± standard deviation) obtained from the pQCT analysis at the diaphyseal 40 % measure point of femur from male foetuses exposed to PCB118 and PCB153. Results from tissue analysis of PCB concentration in the different groups are presented in table 1. Differences were considered significant at 0.0083 (ANCOVA with Bonferroni/Dunn as post hoc). Values sharing the same superscript differ significantly from each other. BMC = Bone Mineral Content, BMD = Bone Mineral Density, CSA = Cross Sectional Area.

| | **Foetus Male (N)** | Reference (17) | Control (12) | PCB118 (17) | PCB153 (10) |
|---|---|---|---|---|---|
| **Diaphysis 40 %** | Total BMC (mg/mm) | 54.0 ± 14.7 | 60.8 ± 8.1 | 54.9 ± 9.9 | 58.8 ± 10.3 |
| | Total BMD (mg/cm$^3$) | 462 ± 83 | 489 ± 75 | 504 ± 114 | 554 ± 62 |
| | Total CSA (mm$^2$) | 117 ± 24 | 126 ± 17 | 112 ± 22 | 106 ± 13 |
| | Cortical BMC (mg/mm) | 26.6 ± 11.6 | 33.5 ± 10.7 | 31.2 ± 9.2 | 34.2 ± 7.8 |
| | Cortical BMD (mg/cm$^3$) | 827 ± 44$^{a.b}$ | 872 ± 46$^{a}$ | 860 ± 42 | 870 ± 22$^{b}$ |
| | Cortical CSA (mm$^2$) | 31.6 ± 12.3 | 37.9 ± 10.6 | 36.0 ± 9.2 | 39.1 ± 8.0 |
| | Cortical thickness (mm) | 0.89 ± 0.33 | 1.05 ± 0.32 | 1.09 ± 0.36 | 1.19 ± 0.22 |
| | Periosteal circumference (mm) | 38.1 ± 4.0 | 39.7 ± 2.6 | 37.3 ± 3.9 | 36.4 ± 2.3 |
| | Endosteal circumference (mm) | 32.5 ± 3.7 | 33.0 ± 3.4 | 30.5 ± 5.3 | 28.9 ± 1.9 |
| | Moment of resistance (mm$^3$) | 82.4 ± 38.9 | 109 ± 35 | 91.8 ± 28.0 | 97.1 ± 27.2 |
| | Marrow cavity (mm$^2$) | 85.2 ± 19.5$^{d}$ | 87.7 ± 19.3$^{c}$ | 76.1 ± 23.7 | 66.9 ± 8.9$^{c.d}$ |

**Foetus female**

**Metaphysis 18 %**

Total BMD was 17 % higher in the PCB153 group (N=14) compared to the reference group (N=8), P<0.0083).

Trabecular CSA was 19 % lower in the PCB153 group (N=14) than in the control group N=16, P<0.0083) (table 8).

**Table 8.** Results (means ± standard deviation) obtained from the pQCT analysis at the metaphyseal measure point of femur from female foetuses exposed to PCB118 and PCB153. Results from tissue analysis of PCB concentration in the different groups are presented in table 2. Differences were considered significant at 0.0083 (ANCOVA with Bonferroni/Dunn as post hoc). Values sharing the same superscript differ significantly from each other. BMC = Bone Mineral Content, BMD = Bone Mineral Density, CSA = Cross Sectional Area.

| | **Foetus Female (N)** | Reference (8) | Control (16) | PCB118 (8) | PCB153 (14) |
|---|---|---|---|---|---|
| **Metaphysis 18 %** | Total BMC (mg/mm) | 46.3 ± 9.8 | 53.1 ± 12.5 | 47.5 ± 7.0 | 55.2 ± 8.7 |
| | Total BMD (mg/cm$^3$) | 402 ± 47$^{a}$ | 428 ± 59 | 435 ± 73 | 485 ± 67$^{a}$ |
| | Trabecular BMC (mg/mm) | 9.99 ± 1.38 | 12.4 ± 4.6 | 9.70 ± 2.2 | 9.93 ± 2.77 |
| | Trabecular BMD (mg/cm$^3$) | 175 ± 33 | 205 ± 66 | 202 ± 61 | 214 ± 69 |
| | Total CSA (mm$^2$) | 115 ± 23 | 124 ± 20.4 | 110 ± 9 | 114 ± 11 |
| | Trabecular CSA (mm$^2$) | 59.1 ± 13.5 | 61.4 ± 12.9$^{b}$ | 50.4 ± 11.5 | 48.2 ± 11.0$^{b}$ |
| | Periosteal circumference (mm) | 37.9 ± 3.9 | 39.3 ± 3.3 | 37.1 ± 1.5 | 37.8 ± 1.9 |

**Diaphysis 60 %**

Total BMD was 15 % higher in the PCB153 group (N=14) compared, both to the reference group (N=8) and to the PCB118 group (N=8, P<0.0083).

Cortical BMD was 5 % higher in the PCB153 group (N=14) compared to the PCB118 group (N=8, P<0.0083).

Cortical thickness was 24 % higher in the PCB153 group (N=14) both compared to the reference group (N=8) and to the PCB118 group (N=8, P<0.0083).

**Table 9.** Results (means ± standard deviation) obtained from the pQCT analysis at the diaphyseal 60 % measure point of femur from female foetuses exposed to PCB118 and PCB153. Results from tissue analysis of PCB concentration in the different groups are presented in table 2. Differences were considered significant at 0.0083 (ANCOVA with Bonferroni/Dunn as post hoc). Values sharing the same superscript differ significantly from each other. BMC = Bone Mineral Content, BMD = Bone Mineral Density, CSA = Cross Sectional Area.

| | **Foetus Female (N)** | Reference (8) | Control (16) | PCB118 (8) | PCB153 (14) |
|---|---|---|---|---|---|
| **Diaphysis 60 %** | Total BMC (mg/mm) | 54.2± 12.9 | 59.4± 12.1 | 52.3± 6.8 | 62.3± 9.0 |
| | Total BMD (mg/cm$^3$) | 579± 71$^{a}$ | 613± 77 | 581± 73$^{b}$ | 682± 81$^{a.b}$ |
| | Total CSA (mm$^2$) | 93.0± 18.2 | 96.9± 17.0 | 90.1± 8.2 | 91.6± 9.8 |
| | Cortical BMC(mg/mm) | 35.9± 12.3 | 41.5± 11.4 | 34.3± 6.7 | 46.1± 9.9 |
| | Cortical BMD (mg/cm$^3$) | 902± 43 | 921± 39 | 897± 33$^{c}$ | 945± 39$^{c}$ |
| | Cortical CSA (mm$^2$) | 39.4± 12.7 | 44.8± 11.4 | 38.1± 6.3 | 48.5± 9.1 |
| | Cortical thickness (mm) | 1.30± 0.40$^{d}$ | 1.49± 0.36 | 1.30± 0.27$^{e}$ | 1.71± 0.35$^{d.e}$ |
| | Periosteal circumference (mm) | 34.0± 3.5 | 34.8± 3.1 | 33.6± 1.5 | 33.9± 1.8 |
| | Endosteal circumference (mm) | 25.9± 2.6 | 25.4± 3.1 | 25.5± 2.6 | 23.1± 2.7 |
| | Moment of resistance ( mm$^3$) | 84.7± 34.2 | 96.1± 34.0 | 76.5± 14.7 | 98.9± 25.9 |
| | Marrow cavity (mm$^2$) | 53.7± 10.8 | 52.2± 12.1 | 52.1± 10.4 | 43.1± 10.2 |

**Diaphysis 50 %**

The PCB 153 group (N=14) had 15 % higher total BMD, 4 % higher cortical BMD and 20 % greater cortical thickness than the control group (N=8, P<0.0083)
Endosteal circumference and marrow cavity was 13 % and 24 % lower, respectively in the PCB153 group (N=14) compared to the control group (N=8, P<0.0083).

**Table 10.** Results (means ± standard deviation) obtained from the pQCT analysis at the diaphyseal 50 % measure point of femur from female foetuses exposed to PCB118 and PCB153. Results from tissue analysis of PCB concentration in the different groups are presented in table 2. Differences were considered significant at 0.0083 (ANCOVA with Bonferroni/Dunn as post hoc). Values sharing the same superscript differ significantly from each other. BMC = Bone Mineral Content, BMD = Bone Mineral Density, CSA = Cross Sectional Area.

| | **Female (N)** | Reference (8) | Control (16) | PCB118 (8) | PCB153 (14) |
|---|---|---|---|---|---|
| **Diaphysis 50 %** | Total BMC (mg/mm) | 52.2± 12.6 | 53.1± 10.9 | 50.5± 8.7 | 57.6± 8.6 |
| | Total BMD (mg/cm$^3$) | 603± 70 | 559± 63[a] | 594± 102 | 660± 75[a] |
| | Total CSA (mm$^2$) | 86.0± 17.1 | 95.0± 16.5 | 85.2± 8.1 | 87.3± 8.2 |
| | Cortical BMC (mg/mm) | 35.4± 11.7 | 35.1± 10.2 | 34.5± 7.2 | 42.3± 9.4 |
| | Cortical BMD (mg/cm$^3$) | 912± 54 | 905± 35[b] | 910± 45 | 948± 36[b] |
| | Cortical CSA (mm$^2$) | 38.4± 11.9 | 38.5± 10.1 | 37.7± 6.5 | 44.3± 8.7 |
| | Cortical thickness (mm) | 1.33± 0.35 | 1.26± 0.32[c] | 1.33± 0.27 | 1.58± 0.32[c] |
| | Periosteal circumference (mm) | 32.7± 3.5 | 34.4± 3.0 | 32.7± 1.6 | 33.1± 1.6 |
| | Endosteal circumference (mm) | 24.4± 2.0 | 26.5± 3.0[d] | 24.3± 2.6 | 23.1± 2.2[d] |
| | Moment of resistance (mm$^3$) | 79.5± 32.6 | 87.3± 30.1 | 75.1± 13.5 | 92.4± 21.3 |
| | Marrow cavity (mm$^2$) | 47.6± 7.5 | 56.5± 12.9[e] | 47.6± 10.3 | 42.9± 8.35[e] |

**Diaphysis 40 %**

No significant differences were found at this measure point (table 11).

**Table 11.** Results (means ± standard deviation) obtained from the pQCT analysis at the diaphyseal 60 % measure point of femur from female foetuses exposed to PCB118 and PCB153. Results from tissue analysis of PCB concentration in the different groups are presented in table 2. Differences were considered significant at 0.0083 (ANCOVA with Bonferroni/Dunn as post hoc). BMC = Bone Mineral Content, BMD = Bone Mineral Density, CSA = Cross Sectional Area.

| | **Foetus Female (N)** | Reference (8) | Control (16) | PCB118 (8) | PCB153 (14) |
|---|---|---|---|---|---|
| **Diaphysis 40 %** | Total BMC (mg/mm) | 45.3± 10.2 | 50.3± 11.7 | 45.0± 5.9 | 52.6± 7.7 |
| | Total BMD (mg/cm$^3$) | 445± 53 | 447± 60 | 459± 75 | 505± 62 |
| | Total CSA (mm$^2$) | 101± 18 | 112± 18 | 99.0± 11.9 | 104± 10 |
| | Cortical BMC (mg/mm) | 23.8± 10.6 | 26.0± 9.1 | 22.4± 6.5 | 30.9± 7.0 |
| | Cortical BMD (mg/cm$^3$) | 827± 54 | 845± 38 | 837± 45 | 871± 37 |
| | Cortical CSA (mm$^2$) | 28.2± 12.1 | 30.4± 9.5 | 26.5± 6.6 | 35.2± 7.1 |
| | Cortical thickness (mm) | 0.84± 0.35 | 0.88± 0.27 | 0.83± 0.25 | 1.08± 0.22 |
| | Periosteal circumference (mm) | 35.5± 3.4 | 37.4± 3.1 | 35.2± 2.1 | 36.2± 1.7 |
| | Endosteal circumference (mm) | 30.2± 2.1 | 31.9± 2.9 | 30.0± 3.3 | 29.4± 2.1 |
| | Moment of resistance (mm$^3$) | 74.4± 38.3 | 84.4± 32.2 | 63.7± 17.7 | 89.4± 19.3 |
| | Marrow cavity (mm$^2$) | 72.9± 9.8 | 81.6± 14.9 | 72.6± 15.7 | 69.1± 10.0 |

**Adult female**

### Metaphysis 4.8 %

No significant differences were found at this measure point (table 12).

**Table 12.** Results (means ± standard deviation) obtained from the pQCT analysis at metaphyseal measure point of femur from gestating ewes exposed to PCB118 and PCB153. Results from tissue analysis of PCB concentration in the different groups are presented in table 3. Differences were considered significant at 0.0083 (ANCOVA with Bonferroni/Dunn as post hoc). BMC = Bone Mineral Content, BMD = Bone Mineral Density, CSA = Cross Sectional Area.

| | **Adult Female (N)** | Reference (14) | Control (14) | PCB118 (14) | PCB153 (11) |
|---|---|---|---|---|---|
| **Metaphysis 4.8 %** | Total BMC (mg/mm) | 379 ± 39 | 390 ± 56 | 373 ± 38 | 382 ± 38 |
| | Total BMD (mg/cm$^3$) | 534 ± 70 | 538 ± 48 | 526 ± 44 | 536 ± 50 |
| | Trabecular BMC (mg/mm) | 69.9 ± 17.5 | 61.9 ± 22.9 | 68.2 ± 20.3 | 72.4 ± 17.2 |
| | Trabecular BMD (mg/cm$^3$) | 182 ± 38 | 161 ± 45 | 173 ± 43 | 185 ± 39 |
| | Total CSA (mm$^2$) | 715 ± 61 | 725 ± 90 | 710 ± 62 | 715 ± 53 |
| | Trabecular CSA (mm$^2$) | 388 ± 79.1 | 381 ± 65 | 391 ± 54 | 393 ± 53 |
| | Periosteal circumference (mm) | 94.7 ± 4.0 | 95.3 ± 5.8 | 94.4 ± 4.1 | 94.7 ± 3.5 |

### Diaphysis 50 %

No significant differences were found on this measure point (table 13).

**Table 13.** Results (means ± standard deviation) obtained from the pQCT analysis at diaphyseal 50 % measure point of femur from gestating ewes exposed to PCB118 and PCB153. Results from tissue analysis of PCB concentration in the different groups are presented in table 3. Differences were considered significant at 0.0083 (ANCOVA with Bonferroni/Dunn as post hoc). BMC = Bone Mineral Content, BMD = Bone Mineral Density, CSA = Cross Sectional Area.

| | **Adult Female (N)** | Reference (14) | Control (14) | PCB118 (14) | PCB153 (11) |
|---|---|---|---|---|---|
| **Diaphysis 50 %** | Total BMC (mg/mm) | 350 ± 29 | 354 ± 42 | 350 ± 37 | 352 ± 38 |
| | Total BMD (mg/cm$^3$) | 684 ± 76 | 701 ± 55 | 685 ± 64 | 670 ± 41 |
| | Total CSA (mm$^2$) | 517 ± 60 | 507 ± 66 | 512 ± 44 | 526 ± 51 |
| | Cortical BMC (mg/mm) | 314 ± 28 | 318 ± 39 | 314 ± 37 | 314 ± 34 |
| | Cortical BMD (mg/cm$^3$) | 1352 ± 24 | 1367 ± 20 | 1354 ± 22 | 1353 ± 25 |
| | Cortical CSA (mm$^2$) | 232 ± 19 | 233 ± 28 | 232 ± 25 | 232 ± 23 |
| | Cortical thickness (mm) | 3.33 ± 0.32 | 3.36 ± 0.29 | 3.33 ± 0.35 | 3.27 ± 0.23 |
| | Periosteal circumference (mm) | 80.5 ± 4.7 | 79.6 ± 5.2 | 80.1 ± 3.5 | 81.2 ± 3.8 |
| | Endosteal circumference (mm) | 59.6 ± 5.9 | 58.5 ± 5.0 | 59.2 ± 4.0 | 60.7 ± 3.4 |
| | Moment of resistance (mm$^3$) | 2176 ± 285 | 2147 ± 367 | 2118 ± 304 | 2185 ± 356 |
| | Marrow cavity (mm$^2$) | 285 ± 58 | 274 ± 46 | 280 ± 38 | 294 ± 34 |

### Diaphysis 40 %

No significant differences were found on this measure point (table 14).

**Table 14.** Results (means ± standard deviation) obtained from the pQCT analysis at diaphyseal 40 % measure point of femur from gestating ewes exposed to PCB118 and PCB153. Results from tissue analysis of PCB concentration in the different groups are presented in table 3. Differences were considered significant at 0.0083 (ANCOVA with Bonferroni/Dunn as post hoc). BMC = Bone Mineral Content, BMD = Bone Mineral Density, CSA = Cross Sectional Area.

| | **Adult Female (N)** | Reference (14) | Control (14) | PCB118 (14) | PCB153 (11) |
|---|---|---|---|---|---|
| **Diaphysis 40 %** | Total BMC (mg/mm) | 350 ± 35 | 351.9 ± 36 | 346 ± 25 | 351 ± 27 |
| | Total BMD (mg/cm$^3$) | 622 ± 71 | 635 ± 51 | 619 ± 48 | 607 ± 41 |
| | Total CSA (mm$^2$) | 568 ± 58 | 556 ± 64 | 560 ± 47 | 579 ± 53 |
| | Cortical BMC (mg/mm) | 311 ± 34 | 315 ± 34 | 308 ± 25 | 312 ± 24 |
| | Cortical BMD (mg/cm$^3$) | 1308 ± 23 | 1319 ± 23 | 1319 ± 24 | 1314 ± 26 |
| | Cortical CSA (mm$^2$) | 237 ± 26 | 239 ± 24 | 233 ± 17 | 237 ± 18 |
| | Cortical thickness (mm) | 3.21 ± 0.38 | 3.26 ± 0.28 | 3.16 ± 0.23 | 3.15 ± 0.21 |
| | Periosteal circumference (mm) | 84.3 ± 4.4 | 83.5 ± 4.8 | 83.8 ± 3.5 | 85.2 ± 3.8 |
| | Endosteal circumference (mm) | 64.2 ± 5.5 | 63.0 ± 5.0 | 64.0 ± 4.1 | 65.5 ± 4.0 |
| | Moment of resistance (mm$^3$) | 2258 ± 352 | 2228 ± 366 | 2219 ± 250 | 2258 ± 275 |
| | Marrow cavity (mm$^2$) | 330 ± 57 | 317 ± 51 | 327 ± 43 | 342 ± 44 |

## Biomechanics Three Point Bending Test

### Male and female foetuses

Due to methodological problems the results from the mechanical testing of the foetus bones are not presented

### Adult female

No significant differences found (table 15).

**Table 15.** Results (means ± standard deviation) obtained from the three point bending test at diaphyseal 50 % point of femur from gestating ewes exposed to PCB118 and PCB153. Results from tissue analysis of PCB concentration in the different groups are presented in table 3. Differences were considered significant at 0.0083 (ANCOVA with Bonferroni/Dunn as post hoc).

| | **Adult Female (N)** | Reference (14) | Control (14) | PCB118 (14) | PCB153 (11) |
|---|---|---|---|---|---|
| **Biomechanics 50 %** | Displacement at failure (mm) | 6.4 ± 1.7 | 6.6 ± 1.8 | 7.0 ± 1.1 | 7.2 ± 1.6 |
| | Load at failure (kN) | 5.49 ± 0.68 | 5.73 ± 0.99 | 5.59 ± 1.04 | 5.37 ± 0.45 |
| | Energy at failure (kN*mm) | 12.8 ± 2.1 | 14.6 ± 4.5 | 13.1 ± 3.2 | 13.3 ± 2.1 |
| | Max Stiffness (kN/mm) | 2.01 ± 0.43 | 2.31 ± 0.50 | 2.09 ± 0.43 | 1.94 ± 0.41 |

# DISCUSSION

The four major findings in this study were;

*i)* an indication that male and female within the foetus group responded differently to the treatment;

*ii)* that major change were on the cortical and not on the trabecular bone;

*iii)* that most changes were connected to the groups with the highest level of PCB153 exposure;

*iv)* that the PCB-exposure did not cause any observable effects on bone tissue in the ewes.

In table 16 the significant results are summarized and comparison is also made between the significant results from a study on goat offspring (see introduction page 10) [4].

The foetus female group had higher number of variables that differed between treatment groups compared to the foetus male groups, 7 vs. 3. The changes were seen on the 18 % and 40 % measure points for the foetus male group and 18 %, 50 % and 60 % for the foetus female group. The two variables; cortical BMD and marrow cavity differed in a similar pattern in the male and female groups (table 16). The bone length was different in the female group with the control group having smaller length compared to the PCB118 and the PCB153 groups.

Trabecular bone is more metabolic active than cortical bone and then effects of stressors would be expected to be more pronounced on trabecular bone. In contrast to results from earlier studies [20] on bone the major changes in this study were primarily seen on the cortical measure points 40 %, 50 % and 60 % and not on the 18 % trabecular measure point.

Because of the complex exposure scenario it is not possible to identify the compound causing the effects. All changes except those on trabecular BMC (foetus male 18 %) were in some way coupled with the PCB153 group. This treatment group was the most “clean” group with the lowest contamination from the other PCB congener (PCB118).
At the 50% diaphyseal measure point in the foetus female group, the observed difference between the control group and the PCB153 group indicate increased bone formation (by stimulation of the osteoblastic cells) or/and decreased bone resorption (by inhibition of the osteoclastic cells) on the inside of the cortical bone but no change on the outside (fig.8). The femur marrow cavity was 24 % smaller and cortex was 20 % thicker in the PCB153 group than in the control group. These results differ from the description of normal diametric growth at the diaphyseal part of femur bone in humans, that is described as formation on the outside and resorption on the inside [11].

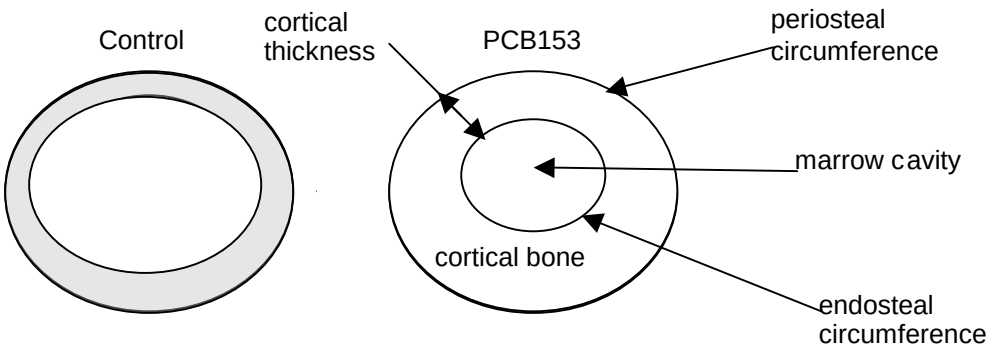


**Figure 8.** A schematic cross section of femur bone (diaphyseal measure point 50 % from control foetuses and foetuses exposed to PCB153).

The results in the present study are in accordance with the established belief that young individuals are more susceptible than adults to chemical exposure.

## Environmental relevant concentration

Concentrations of PCBs found in wild animals are a bit lower than the levels in this study. The average sum of the two PCB congeners in the PCB153 groups were 48 µg/g lipid in the foetuses and in the adults as much as 60 µg/g lipid. Grey seals from the waters of Scotland have been reported to have a blubber concentration of around 3 µg/g lipid [40]. In yearlings to 3 years old grey seals from the Baltic Sea the total concentration of PCB in blubber over the years 2000-2005 was 17 µg/g lipid (median value) [41]. Seals have in general much more fat per kg body weight than sheep and thus a higher ability to dilute the PCBs in fat tissue. In the current study difference in the foetus male group was observed between the low exposed control group (~3.6 µg of PCB118 + PCB153/g lipid) and the non treated reference group on the variable cortical BMD. The difference was in the same level as the difference found between the PCB153 and the reference groups (table 16).

## Comparable studies

Other studies with comparable design and endpoints have to my best knowledge not been undertaken. The study on sheep exposed to PCB118 and PCB153 *in utero* and during lactation (see introduction page10), [30] comes closest. The study involves the same ewes as the current study. The exposure scenario is similar with lambs (60 females and 60 males) exposed to PCB118 and 153 *in utero* from day one of gestation and then after birth during lactation for 21 weeks. Lambs were then euthanised at the age of 21 weeks. Differences were found only on the theoretical variables for bone strength, moment of resistance and polar moment of inertia. The diverging results might in part be because the statistical analysis of the results was performed separately for the sexes in the present study but not in the earlier study. Treating the male and female as one group might create a larger variation within the groups and thereby hiding possible significant differences.

The marrow cavity was smaller in the femur from foetuses in the PCB153 group compared to the control and reference groups. A similar result was found in goat perinataly exposed to PCB153 [4] (see introduction page 10), (table 16). The increasing trabecular BMD in the goat offspring exposed to PCB153 suggests an estrogenic activity (*in vivo*) of this PCB congener. The findings of the current study do not contradict this idea although no effects on trabecular BMD were found. The indicated bone formation/inhibition of bone resorption in the current study as visualised in figure 8 could be interpreted as an estrogenic effect. It could not be elucidated from which of the two congeners the effect was arising.

## Biomechanics

The result from the biomechanical testing on femur from the ewes revealed no significant differences between the different groups. Since no effects of PCB exposure were seen on the composition of the bones the absence of effects also in the three point bending test was to be expected.

Table 16. Overview of results from pQCT measurements in the present study contra the goat study by Lundberg et al [4] showing differences between treatments groups on different measure points . Direction of change is indicated by arrows, the results were similar on the variable marrow cavity indicated with dotted lines. Tissue concentration of PCB for each treatment group involved in the present study is presented in table 1 and 2. Measurements from the compared goat study were performed on female goat offspring euthanatized at the age of 9 month, the suckling period lasted for 6 weeks. Ewes were treated from day 60 of gestation until delivery with 98 μg/kg body wt/day of PCB153, controls were treated with corn oil. Number of animals was 7 for both groups. BMC = Bone Mineral Content, BMD = Bone Mineral Density, CSA = Cross Sectional Area, ^Control group did not have Gaussian Distribution, ^^percent of the total bone length from the proximal tip of the bone both 9 % and 18 % are considered to be trabecular measure points.

| **Differing variables** | **Foetus Male** | | **Foetus Female** | | | **Goat 9 month** [4] | |
|---|---|---|---|---|---|---|---|
| | **18 %** | **40 %** | **18 %** | **50 %** | **60 %** | **9 %^^** | **18%^^** |
| Trabecular BMC | 118↓ Vs. Ref<br>118↓ Vs. Con | | | | | | |
| Trabecular BMD | | | | | | 153↑ Vs Con | |
| Trabecular CSA | | | 153↓ Vs Con | | | | |
| Cortical BMD | | 153↑ Vs Ref<br>Con↑ Vs Ref | | 153↑ Vs 118 | 153↑ Vs Con | | |
| Cortical thickness | | | | 153↑ Vs Ref<br>153↑ Vs 118 | 153↑ Vs Con | | |
| Total CSA | | | | | | | 153↓ Vs Con |
| Total BMD | | | 153↑ Vs Ref | 153↑ Vs Ref<br>153↑ Vs 118 | 153↑ Vs Con | | |
| Endosteal circumference | | | | | 153↓ Vs Con | | |
| Marrow cavity | | 153↓ Vs Ref<br>153↓ Vs Con^ | | | 153↓ Vs Con | 153↓ Vs Con | 153↓ Vs Con |
| Moment of resistance | | | | | | | 153↓ Vs Con |

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