TRABECULAR, BUT NOT CORTICAL, BONE TISSUE IS AFFECTED BY SHORT-TIME EXPOSURE TO DIOXIN IN MALE SPRAGUE/DAWLEY RATS.

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Introduction
Persistent organic pollutants (POPs) are known environmental pollutants. Studies on free-ranging wild animals, including East Greenland polar bears *(Ursus maritimus)* [1] and Baltic grey seals *(Halichoerus grypus)* [2], exposed to a mixture of persistent organic pollutants, have revealed decreased bone mineral density (BMD). Also the results from two epidemiological studies on Swedish professional fishermen and their wives, indicated a correlation between POP exposure through fatty fish and osteoporotic fractures [3, 4]. In addition, experimental chronic and sub-chronic studies in rats have demonstrated that high affinity aryl hydrocarbon receptor (AhR) ligands such as TCDD and the dioxin-like PCB congener, 3,3',4,4',5-pentachlorobiphenyl (PCB126), impair bone tissue composition and strength[5-7]. The main purpose of this pilot study was to investigate whether short-time exposure to dioxin affects bone tissue or not.

Material and Methods
Two months old, male Sprague/Dawley (SD) rats (200g) were injected intraperitoneally with 50µg 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD)/kg bw in corn oil and tibiae were excised 5 days following exposure. Bone tissue composition and dimensions were determined by peripheral quantitative computed tomography (pQCT) on excised tibiae as earlier described [8, 9]. Cortical parameters (total cross-sectional area (mm^2), cortical thickness (mm), cortical bone mineral content (mg/mm), cortical bone mineral density (mg/cm^3) and moment of resistance (mm^3)) were determined by mid-diaphyseal scans of tibiae, while trabecular parameters (total cross-sectional area (mm^2) trabecular area (mm^2), trabecular bone mineral content (mg/mm) and trabecular bone mineral density (mg/cm^3)) were determined by metaphyseal scans. The results obtained were evaluated by ANOVA (one-way analysis of variance) and by principal component analysis (PCA). PCA was used to generate factors and factor scores. Significance values were given with $p < 0.05$ regarded as significant.

Results and discussion
The results show that short time exposure to TCDD has a significant impact on the metaphyseal part of tibia, but not on the diaphyseal part (Table 1). Statistical evaluation using ANOVA revealed a significant decrease in trabecular area of the metaphysis in TCDD exposed rats vs non-exposed rats ($9.4\ mm^2 \pm 0.5$, n=9 vs $11.5\ mm^2 \pm 0.2$. n=9, $p<0.01$). PCA of the trabecular measurements revealed one factor being significantly reduced in the exposed animals ($p<0.01$, fig 1). The factor included trabecular bone mineral content and trabecular area. The factor was still significantly reduced by exposure after adjustment for body weight ($p<0.05$). In conclusion, short-time exposure to TCDD affected the mineralization of the trabecular, but not cortical bone of male SD rats, indicating a more rapid response in the trabecular tissue compared to cortical tissue. The underlying cause for this observation might be due to the fact that the metabolic rate of trabecular bone is much greater than that of cortical bone. Since TCDD is a potent modulator both of metabolic and endocrine pathways it is tempting to speculate that skeletal sites containing more trabecular bone will be affected more rapidly by TCDD than cortical bone. This effect was seen independently of body weight, suggesting trabecular bone tissue as a sensitive marker of TCDD exposure.

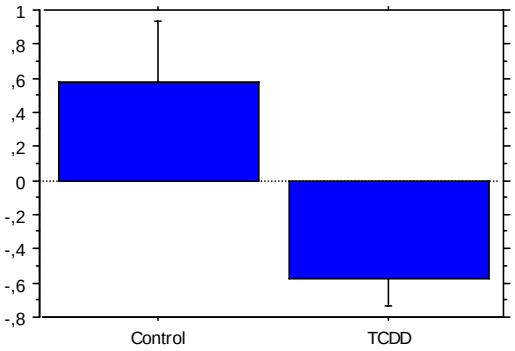

Fig 1. Mean values of the factor scores.

Table 1. Results obtained from pQCT –measurements of tibia from male rats given a single injection of 50µg 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD)/ kg bw**.** Controls were injected with corn oil.

Endpoints	Control N = 9			Exposed N = 9		
Final body weight (g)	238,3	±	17,6	226,9	±	18,9
Length of tibia (mm)	35,2	±	0,7	35,3	±	0,8
DIAPHYSIS						
Total cross-sectional area (mm^2)	5,4	±	0,5	5,2	±	0,4
Cortical thickness (mm)	0,43	±	0,01	0,42	±	0,02
Cortical bone mineral content (mg/mm)	3,7	±	0,2	3,5	±	0,2
Cortical bone mineral density (mg/cm^3)	1227,2	±	16,8	1227,7	±	16.0
Moment of resistance (mm^3)	2,3	±	0,2	2,2	±	0,2
METAPHYSIS						
Total cross-sectional area (mm^2)	20,2	±	2,4	18,9	±	2,7
Trabecular area (mm^2)	11,5	±	1,6	9,4	±	0,7*
Trabecular bone mineral content (mg/mm)	2,6	±	0,4	2,3	±	0,3
Trabecular bone mineral density (mg/cm^3)	222,9	±	9,6	242,5	±	31,7

Values are mean ± SD?
N = number of individuals
* $p<0.01$ compared to control.

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