PERINATAL EXPOSURE TO A MIXTURE OF PERSISTENT POLLUTANTS BASED ON BLOOD PROFILES OF ARCTIC POPULATIONS AFFECTS BONE PARAMETERS IN 35 DAYS OLD RATS

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Introduction

Environmental pollution of Arctic regions is a public concern. Arctic inhabitants are a high-risk group regarding health effects of environmental toxicants because of their high consumption of contaminated fish and wildlife. Developing fetuses and newborn infants may be particularly vulnerable to the effects of exposure to persistent organic pollutants (POPs) and toxic metals. Developmental exposure to environmental pollutants affects a wide range of clinical and biochemical parameters. Disturbance in skeletal growth, an integral component of somatic development, is a novel area in the toxicity of POPs. Low-dose prenatal exposure to TCDD caused a variety of harmful effects in rat long bones[1]. In adult rats, TCDD exposure caused inhibited bone growth and lowered biomechanical properties of tibia[2] and exposure to the dioxin-like PCB-congener 3,3,4,4,5-pentachlorobiphenyl has been associated with a decreased strength and collagen concentration of humerus[3].
The aim of this study was to investigate effects of perinatal exposure to a mixture of PCBs, organochlorines and methyl mercury based on blood levels of Canadian Arctic populations on skeletal development in rat pups.

Methods and Materials

Male and female Sprague-Dawley rats were allowed to acclimatize for at least three weeks. Mated and confirmed-pregnant females were randomly assigned to the vehicle control group (corn oil), positive control group (15 mg/kg/day Aroclor 1254) and three dose groups: low (0.05 mg/kg/day), medium (0.5 mg/kg/day) and high (5 mg/kg/day) of the mixture that was based upon relative concentrations in the blood of Canadian Inuit populations (Arctic mixture). This mixture was composed of 14 PCB congeners, 12 different organochlorines and methyl mercury chloride (21%, 41% and 38% by weight respectively). Pregnant rats were dosed orally from gestation day 1 to weaning (postnatal day 22); the pups were never dosed directly. On PND 4 the litter sizes were adjusted to four males and four females. One male and one female from each litter were sacrificed as juvenile (PND 35 – results presented here), as a young adult (PND 75) or as a mature adult (PND 350). The animals were sacrificed by decapitation. The right femur was dissected and stored in Ringer solution at -20ºC until analysis. The bone length was measured using an electronic sliding calliper. The femur was scanned at points distanced 20% (metaphysis) and 50% (diaphysis) of length from the distal end of femur with a peripheral quantitative computed tomography (pQCT) system (Stratec XCT 960A, Birkenfeld, Germany) with a voxel size of 0.148 mm^3. At the metaphyseal pQCT scan the cross-sectional area was analysed and trabecular volumetric bone mineral density (BMD) was determined at the area defined as 45% of the total area. A 0.7 cm^{-1} attenuation threshold was set to define cortical bone in femur diaphysis. Cortical volumetric BMD, cortical area, periosteal circumference, endoosteal circumference, cortical thickness, polar moment of inertia and polar moment of resistance were determined at the diaphyseal scan of femur. The differences among groups were examined using the Kruskal-Wallis one-way analysis of variance by ranks. In cases of statistically significant ($p < 0.05$) differences each group was tested against the corresponding vehicle control groups using the Mann-Whitney U test. A significance level of $p<0.05$ was chosen.

Results and Discussion

At 35 days of age body weight was significantly decreased in male and female pups exposed to high dose of Arctic mixture (Table 1). The length of the femur was significantly increased in female pups exposed to low and medium doses and in male pups exposed to medium dose of Arctic mixture (Figure 1).

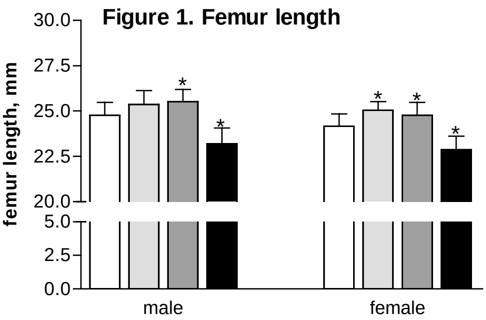
Table 1. Cortical thickness, periosteal circumference, endoosteal circumference and polar moment of resistance in the femoral diaphysis of 35 day old male and female pups after perinatal exposure to mixture of persistent environmental pollutants (Arctic mixture).

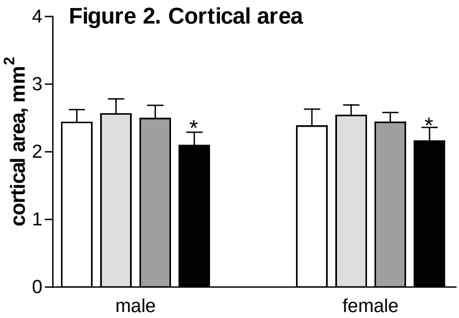
		Dose mg/kg/ day	Body weight, g	Periosteal circumference, mm	Endoosteal circumference, mm	Cortical thickness, mm	Polar moment of resistance, mm^3
male	Vehicle	control	143 ± 17	9.7 ± 0.2	8.0 ± 0.2	0.27 ± 0.02	2.2 ± 0.2
	Arctic mixture	0.05	142 ± 16	9.6 ± 0.4	7.7 ± 0.4	0.30 ± 0.02*	2.2 ± 0.3
		0.5	145 ± 14	9.4 ± 0.4	7.5 ± 0.4*	0.29 ± 0.01	2.2 ± 1.4
		5	110 ± 13*	9.0 ± 0.3*	7.4 ± 0.2*	0.26 ± 0.02	1.7 ± 0.2*
female	Vehicle	control	124 ± 10	9.2 ± 0.5	7.4 ± 0.5	0.29 ± 0.02	2.0 ± 0.3
	Arctic mixture	0.05	129 ± 9	9.1 ± 0.3	7.2 ± 0.3	0.31 ± 0.02*	2.1 ± 0.3
		0.5	127 ± 9	9.1 ± 0.4	7.2 ± 0.5	0.30 ± 0.02	2.0 ± 0.2
		5	99 ± 11*	8.8 ± 0.3	7.1 ± 0.3	0.27 ± 0.02	1.7 ± 0.2*

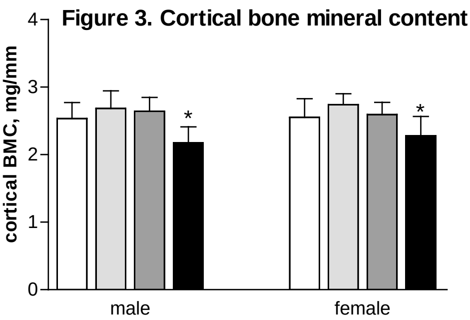
[a]Values represent the mean ± SD of 8 to 10 animals
**p<0.05 compared to corresponding controls (Mann-Whitney U test)*

The length of the femur was decreased both in male and female pups exposed to high dose of Arctic mixture compared to corresponding controls. Cortical area (Figure 2) and bone mineral content (Figure 3) were decreased in male and female pups exposed to high dose of Arctic mixture

compared to controls. In male pups exposed to high dose of Arctic mixture the decreased cortical area was associated with decreased cortical periosteal circumference as well as cortical endoosteal circumference, resulting in no significant changes in cortical thickness (Table 1).
The changes in cortical bone content and the cortical area in male and female pups exposed to high dose of Arctic mixture resulted in a decrease of the polar moment of inertia (Figure 4) and the polar moment of resistance (Table 1) suggesting lower strength of the cortical bone in these groups compared to control pups.







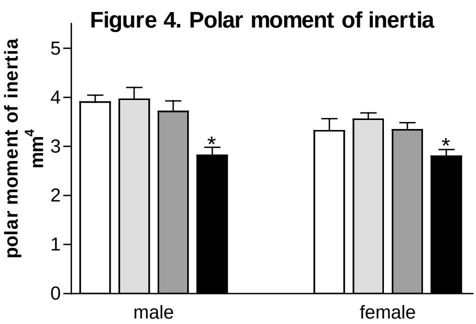


Figure 1-4. Bone length (mm), cortical area (mm^2), cortical bone mineral content (mg/mm) and polar moment of inertia (mm^4) in the femoral diaphysis of 35 day old pups after perinatal exposure to mixture of persistent environmental pollutants (Arctic mixture).
Values represent the mean ± SD of 8 to 10 animals exposed to

- vehicle, - 0.05 mg/kg/day, - 0.5 mg/kg/day and - 5 mg/kg/day of Arctic mixture
**p<0.05 compared to corresponding vehicle controls (Mann-Whitney U test)*

Cortical thickness (Table 1) was significantly increased in male and female pups exposed to low dose of Arctic mixture. Trends of increased cortical area, bone mineral content and polar moment of inertia (Figure 2-4) were observed both in male and female pups exposed to low and medium doses of Arctic mixture, however the values did not reach the statistical significance. Neither trabecular nor cortical BMD were affected by treatments (data not shown).

Conclusions

In the present study, the maternal dose of 5mg/kg/day of Arctic mixture caused decreased body weight, bone length and cortical geometrical parameters in both male and female pups. These data are in agreement with previously reported effects of gestational TCDD exposure on tibial growth and strength in rat pups[1]. It should be noticed, however, that the low-dose maternal exposure to the Arctic mixture resulted in significantly increased bone length and cortical thickness in male and female pups. Thus, an opposite effect was noticed, indicating the stimulatory effect of low dose exposure on these parameters. The chemical analysis of blood levels of the Arctic mixture components are needed to address their role in the observed effects. Additional chemical, biochemical, endocrine and histopathological analysis from pups sacrificed at PND 75 and PND 350 will provide better understanding of the potential health hazards of human exposure to environmental pollutants in Arctic regions.

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