Effects of polychlorinated biphenyls on bone growth

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Introduction
Growth disorders as a result of environmental pollutants (Hoffmann et al., 1996) and the accumulation of polychlorinated biphenyls (PCBs) in the fat tissue have been reported in many studies. It is still unknown, whether the alteration in bone metabolism is a general answer of the organism or there is any direct bone impairment. The aim of our study was to elucidate the influence of different concentrations of PCBs on bone-growth under standardized in vitro conditions.

Materials and methods
Femura and tibiae of 11 days old chicken (White Leghorn) were carefully dissected. The influence of different PCB-concentrations was examined by pair matching (PCB-exposed versus controls (received vehicle only)) in an organ culture. Three concentrations of PCBs (0.6 pg/ml; 0.6 ng/ml; 0.6 µg/ml) were used for bone culture . The added PCB-mixture consisted of 10 congeners: PCB 28; 52; 77; 101; 118; 126; 138; 153; 169; 180. All experiments were repeated to prove results. Additionally we investigated the influence of the stereoscopic structure of PCBs on ossification. Two culture media were used, containing mixtures of coplanar PCBs (PCB 77; 126; 169) and non-coplanar PCBs (PCB 28; 52; 101; 118; 138; 153; 180) in a concentration of 0.6 ng/ml, respectively. After incubation (6 days, 37°C, 5% CO_2) the bones were photographed, fixed in 4% formalin and then embedded in paraffin. At the end of incubation we made controls to check the PCB-content of the media .
Histological analysis: The Ki-67-protein (Gerdes et al., 1991) was immunohistochemically labeled by the monoclonal mouse-anti-Ki-67-protein (rat) dia5055 (dianova Hamburg) antibody. Sections were incubated at 4°C overnight. As second antibody we used the peroxidase-conjugated-goat-anti.mouse antibody (118-036-003, dianova). Immunostaining was detected by the „DAB substrate kit 34062“ (Pierce, Rockford II, USA).

Results and Discussion
Retardation of growth could be shown in all experiments (n = 176 bone pairs; $p<0.05$). The difference of the relative growth (DORG) was greater using PCBs in concentrations of 0.6 pg/ml (9.7%) and 0.6ng/ml (9.5%) than concentration of 0.6 µg/ml (5.3%) [Figure 1; $p < 0,05$]. The observed difference in growth inhibition between the lower concentrations (pg/ml and ng/ml) could not be proved statistically. Addition of non-coplanar PCBs to culture media results in a significant greater growth retardation (DORG: 14.5%) than addition of coplanar PCBs (DORG: 9.2%) [Figure 2; $p < 0,05$]. Histological results: All investigated bones (n = 18) were

Figure 1

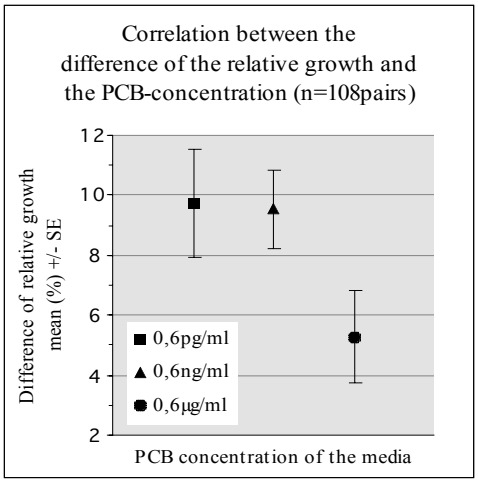
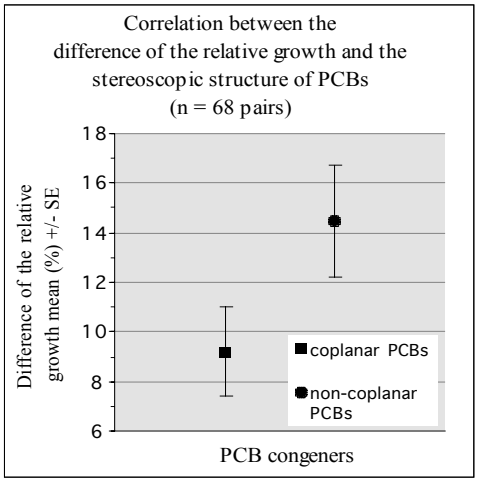


Figure 2



immunopositive for the Ki-67-protein as an expression of a persistent proliferation. First experiments showed a reduced staining intensity in the growth plate of PCB-exposed bones.
Our findings of a reduced growth of in-vitro culture using media containing PCBs revealed a direct alteration of bone growth. Remarkable is the reverse relationship between PCB-concentration and growth retardation, which is already described for an activation process by PCBs in the liver (Hahn et al., 1996). The elucidation of the molecular mechanism requires more intensive work. The effect of PCBs could not be mediated only by the aryl hydrocarbon receptor (AhR) because bones are more vulnerable to non-coplanar PCBs, which show a weak or no binding to the AhR (Bandiera et al., 1982).

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